

Title: Preservation and Survival of *E. coli* in Well Water Samples Submitted for Routine Analyses

Project I.D.: DNR Project #166

Investigators: William Sonzogni, Jon Standridge (primary contact), and Michelle Bussen – Wisconsin State Laboratory of Hygiene, Environmental Health Division

Period of Contract: 07/01/01 through 06/30/02

Background/Need: Total coliform testing to determine potability of drinking water has been performed routinely on Wisconsin well waters since the beginning of the 20th century. The data derived from this testing has driven the development and continuous improvement of the administrative codes that regulate well construction and maintenance. The quality assurance programs mandated by the United States Environmental Protection Agency associated with laboratory testing for coliforms establish maximum sample holding times. The agency uses published data and expert opinion as the basis for setting the required holding times. Currently the holding time for drinking waters is set at 30 hours. In Wisconsin, the Laboratory of Hygiene (WSLH) did an extensive study that demonstrated coliforms actually survive quite well for up to 48 hours at ambient temperatures in typical Wisconsin well water samples. Subsequent to this study, the USEPA granted an exception for the WSLH to allow compliance testing for total coliforms up to 48 hours after collection. This exception has permitted a high compliance rate for required testing of drinking waters, even from remote areas of the state where delivery to the laboratory can often take two days.

During the mid-nineties, changes in available technology moved the testing of drinking water ahead by a quantum leap. The introduction of enzyme based assays allowed for low cost, rapid testing for *E. coli* simultaneously with total coliform analysis. While the occurrence of total coliforms indicates that a water sample is contaminated, the detection of *E. coli* provides direct unequivocal evidence of fecal contamination. The ability to easily detect *E. coli* has precipitated actual changes and proposed changes in how officials respond to coliform/*E. coli* testing results. This general move towards decision making based on *E. coli* test results, creates a need for understanding whether or not *E. coli* survives as well as the total coliform group while samples are in transit to the laboratory. Previously published work in this area is incomplete since it focused primarily on surface waters and studied a limited array of temperatures and storage times. The work described in this report provides information to more completely understand the issue of *E. coli* survival in water samples. The topic of establishing holding times for water samples submitted for *E. coli* testing is extremely important and timely. If *E. coli* dies off during transit to the laboratory, unsafe water supplies could be classified as safe. Data is needed for making sound decisions regarding sample preservation requirements.

Objectives: The purpose of this study was to provide data on the survival of *E. coli* in water samples, to be used in setting storage and handling requirements for water samples submitted for Coliform and *E. coli* analysis.

Methods: A variety of surface and groundwaters were collected from southern Wisconsin. While some of the samples had naturally occurring *E. coli*, most required spiking. Triplicate samples were prepared and tested across a matrix of 4 temperatures (4, 10, 20 and 35 degrees C) and 4 time periods (8, 30, 48 and 72 hours). *E. coli* testing

was performed with the IDEXX Laboratory Colilert/ Quantitray 2000 coliform testing system.

Results and Discussion:

All samples including those with very low levels of bacteria can safely be preserved for at least 48 hours if held at 4 degrees C, the temperature usually achieved by shipping samples packed in wet ice. 48-hour package delivery to Madison can easily be achieved from all areas of Wisconsin. Thus, water samples shipped in coolers packed with wet ice could be accurately analyzed up to 48 hours after collection. The data also shows that in all the trials except one, *E. coli* can be preserved for 48 hours when held at 10 degrees C, and all samples can be preserved at 10 degrees C for 30 hours. 10 degree shipping temperatures can be achieved with the use of "blue ice" freezer packs in coolers, which simplifies the shipping process as compared to dealing with wet ice. The current practice of shipping drinking water samples in Styrofoam boxes to hold the temperature at approximately 20 degrees C would be valid for almost all samples for up to 30 hours. The data clearly shows that samples held at 30 degrees C are unsuitable for *E. coli* testing. This finding suggests that samples submitted during the summer months, with no attempt at preservation through cooling would not be suitable for *E. coli* testing.

Conclusions/ Implications:

While sample holding time criteria is in place for drinking water samples submitted for total coliform analysis, currently the USEPA has no guidelines for sample holding times and shipping temperatures for drinking water samples submitted for *E. coli* testing. For surface water samples the guidelines state that samples must be refrigerated in transit and tested within eight hours of collection. The general move in the regulation of drinking water towards decision making based on *E. coli* test results, creates a need for understanding whether or not *E. coli* survives as well as the total coliform group while samples are in transit to the laboratory. The work described in this report provides much of this information. The data provides a strong basis for a decision to expand the allowable storage time of water samples submitted for *E. coli* analysis beyond the current eight hour limit as well as the basis for supporting only one recommended preservation protocol for both surface waters and drinking water samples.

Recommendations: This data suggests that the USEPA established holding time of eight hours for surface water samples submitted for *E. coli* testing, is overly stringent. A change to a maximum holding time of chilled samples for up to 30 hours could easily be supported by the data presented in this study. The data also suggests that the current practice of allowing up to 48 hours for drinking water samples submitted with no attempt to cool the samples may be too lax. A reduction in the time period to 30 hours, or a requirement to ship the samples at less than 10 degrees C, could be supported by the data.

Related Publications:

Bussen, M. and J. Standridge. 2001. Preservation and Survival of *E. coli* in Well Water Samples Submitted for Routine Analysis. Proceedings, 2001 American Waterworks Association Water Quality Technology Conference.

Key Words: *E. coli*, groundwater, sample preservation

Funding: Wisconsin Department of Natural Resources

Final Report: A final report containing more detailed information on this project is available for loan at the Water Resources Institute Library, University of Wisconsin - Madison, 1975 Willow Drive, Madison, Wisconsin 53706 (608) 262-3069.